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## Live cell imaging of low- and non-repetitive chromosome loci using CRISPR-Cas9.

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### Public Summary:

Imaging chromatin dynamics is crucial to understand genome organization and its role in transcriptional regulation. Recently, the RNA-guidable feature of CRISPR-Cas9 has been utilized for imaging of chromatin within live cells. However, these methods are mostly applicable to highly repetitive regions, whereas imaging regions with low or no repeats remains as a challenge. To address this challenge, we design single-guide RNAs (sgRNAs) integrated with up to 16 MS2 binding motifs to enable robust fluorescent signal amplification. These engineered sgRNAs enable multicolour labelling of low-repeat-containing regions using a single sgRNA and of non-repetitive regions with as few as four unique sgRNAs. We achieve tracking of native chromatin loci throughout the cell cycle and determine differential positioning of transcriptionally active and inactive regions in the nucleus. These results demonstrate the feasibility of our approach to monitor the position and dynamics of both repetitive and non-repetitive genomic regions in live cells.

### Scientific Abstract:

Imaging chromatin dynamics is crucial to understand genome organization and its role in transcriptional regulation. Recently, the RNA-guidable feature of CRISPR-Cas9 has been utilized for imaging of chromatin within live cells. However, these methods are mostly applicable to highly repetitive regions, whereas imaging regions with low or no repeats remains as a challenge. To address this challenge, we design single-guide RNAs (sgRNAs) integrated with up to 16 MS2 binding motifs to enable robust fluorescent signal amplification. These engineered sgRNAs enable multicolour labelling of low-repeat-containing regions using a single sgRNA and of non-repetitive regions with as few as four unique sgRNAs. We achieve tracking of native chromatin loci throughout the cell cycle and determine differential positioning of transcriptionally active and inactive regions in the nucleus. These results demonstrate the feasibility of our approach to monitor the position and dynamics of both repetitive and non-repetitive genomic regions in live cells.

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